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11/16/94

Project No. _____

Book No. _____

TITLE Dep Vent / GAPDH / diff primers

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Purpose: Since GAPDH - PCR worked with 3' Thiol primers attempted the same amplification with other available primers, under same conditions

- Dep vent buffer enzyme at 1U and 0.5U
200 pg Template Mg at 2, 3, 4 and 6 mM
200 μ M dNTP
1 μ M primers

did just one of each. primers

* 2697 & 2696 no Lac END & Lac Reverse (100 μ M)
" " " 3'-1 PPT
+ dU " " (10 μ M)

- each primer set 10X RX were made.

Regular	17-20 = 0.5 U	dU	33-36 = 0.5 U	3'-1 PPT	
	21-24 = 1.0 U		37-40 = 1.0 U		25
10x buffer	50	50		50	29
dNTP	10	10		10	
primer 1	5	50		20	
2	5	50		20	
Template	20 (100 pg/ λ)	20		20	
H ₂ O	260	270		330	

450 \rightarrow 45 μ L/RX \leftarrow 450 \leftarrow 450

Added μ mol of	2	3	4	6	mM	
Mg chlorination	1	1	1	1		67-20
	0	0.5	1	2	(100 mM)	10X
	5	4.5	4	3	1120	

- enzyme added individually 0.25 μ L for 0.5 U
0.5 μ L for 1 U

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Witnessed & Understood by me,

Date

Inventor by

Date

Recorded by

R. Schuman

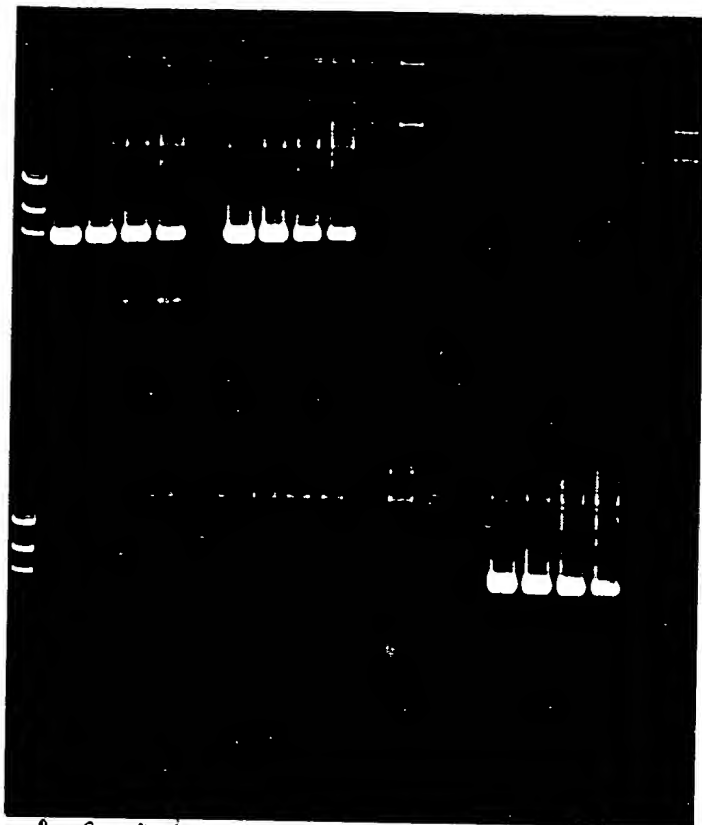
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3g N

Regular

3'-1 PPT

0.5U

2 3 4 6
at 0.52 3 4 6 mol 12g
1U

du primer

Vent +/- exo didn't discriminate between modified and unmodified
 " with du in earlier lines per 2 500 bp Lac 2 never got
 amplified

- samples discarded 12/19/94

Result

- Regular - unmodified, revised, so couldn't say 3'-1 PPT is better than unmodified!

- du certainly has problem with Deep Vent.

- with the amount of product seen with 1U don't know why there is no products with 0.5U & 3'-1 PPT primers

Con of template - ?
 Contamination -
 what are these bands on the top.

- Template / primer - no enzyme controls also have them?

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Date

11/28/94

Invented by

Recorded by

Dr. S. S. S. S. S.

Dat

11/18/94